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EFFECT OF VARIOUS TREATMENTS ON GERMINATION OF DORMANT SEEDS OF *STRELITZIA REGINAE* AIT.

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Abstract

The effects of a wide variety of dormancy-breaking treatments on the germination of *S. reginae* seeds were investigated. Germination was improved after treating the seeds with thiourea, various mercaptans, ascorbic acid and ethrel; by incubation in elevated oxygen concentrations; and by removal of the tissues overlying the embryo in the seed. Treatments which did not improve germination included incubation in elevated carbon dioxide concentrations, leaching with water, various storage conditions, and treatment with urea, indoleacetic acid, gibberellic acid, kinetin, various oxidizing agents, and respiratory inhibitors. Low temperatures had a decidedly harmful effect on both dry and imbibed seeds.

Uittreksel

INVLOED VAN VERSKILLENDE BEHANDELINGS OP DIE ONTKIEMING VAN *STRELITZIA REGINAE* AIT. SADE WAT IN 'N RUSTOESTAND VERKEER

Die invloed van 'n verskeidenheid rusbeëindigende behandelings op die ontkieming van *S. reginae* sade is ondersoek. Ontkieming is verbeter nadat sade behandel is met tio-ureum, verskeie merkaptane, askorbiensuur en ethrel; deur inkubasie in verhoogde suurstofkonsentrasies sowel as na verwydering van die weefsels wat die embrio in die saad oordek. Behandelings wat ontkieming nie verbeter het nie is: inkubasie in verhoogde koolstofdioksiedkonsentrasies, loging van die sade met water, verskeie opbergingstoestande, asook saadbehandeling met ureum, indoolasynsuur, gibberelliensuur, kinetien, verskeie oksideermiddels en respiratoriese inhibeerders. Lae temperature het 'n besliste nadelige uitwerking gehad op beide droë en geïmbibeerde sade.

Introduction

Seeds of the acaulescent species of *Strelitzia* exhibit a condition of innate, relative dormancy (van de Venter & Small, 1974). Evidence has been presented that an inhibitor may be involved in this phenomenon (van de Venter & Small, 1975) but this does not preclude the possibility of contributions by other factors since dormancy can be the result of a combination of factors (Villiers, 1972).

In an attempt to obtain more information on the mechanisms controlling dormancy of *S. reginae* seeds, the preliminary approach of determining the effect of various well-known dormancy-breaking treatments on these seeds was used. A treatment which will promote the germination of dormant seeds can be expected to have some bearing on the operative mechanism and it was envisaged that these experiments would provide guidelines for further research.

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MATERIAL AND METHODS

Seeds of *S. reginae*, harvested from various plants in the East London area, were used. The seeds were pooled and stored under ambient conditions in the laboratory (*ca*. 20 °C). All experiments were conducted on seeds that were less than a year old.

The seeds were sterilized and germinated in petri dishes as previously described (van de Venter & Small, 1974). Due to limited quantity, only 25 seeds per dish represented one replicate. The number of replicates varied between four and eight, depending on the particular experiment.

Unless otherwise mentioned, only scarified seeds were used. Incubation proceeded in the dark at 25 ± 0,5 °C and counts were made after 14 days. The seeds were scarified with emery paper to expose a few square millimeters of endosperm.

Treatment of seeds with various chemicals was accomplished by immersing them (after surface-sterilization) for 48 hours in sterile solutions of these compounds, prior to incubation in petri dishes.

In experiments involving treatments with oxygen and carbon dioxide, seeds were incubated in sterile, 130 mℓ-capacity conical flasks containing 4 mℓ sterile, distilled water. Oxygen and carbon dioxide concentrations were adjusted to required values by injecting specific volumes of these gases through the vaccine caps sealing the flasks. Where an atmosphere of pure oxygen was required, flasks were flushed with this gas for three minutes.

Arcsin transformation of germination percentages was carried out before statistical tests were applied and lowest significant difference (LSD) values calculated according to the ω-procedure of Tukey (Steel & Torrie, 1960).

RESULTS

Effect of light and scarification

Scarified and unscarified seeds were subjected to light and dark treatments in a 2 × 2 factorial design. Light was supplied from cool white fluorescent tubes at an intensity of *ca*. 12 000 lux. Petri dishes of the dark treatment were wrapped in a single layer of aluminium foil.

The results of this experiment (Table 1) show that scarification did not improve germination to a significant extent. However, this treatment improved water absorption by the seeds (Fig. 1). Light had a harmful effect on the germination of unscarified seeds.

Effect of thiourea, urea, various mercaptans and ascorbate

Seeds were soaked in different concentrations of thiourea, urea, reduced glutathione, L(+) cysteine, 2,3-dimercapto-1-propanol (BAL) and L(+) ascorbic acid. All of these compounds, with the exception of urea, promoted germination at certain concentrations (see Table 2).

TABLE 1

Germination percentage of scarified and unscarified seeds of *S. reginae* in light and dark (Arcsin transformed values in brackets)

	Light	Dark	Mean
Scarified seeds	31 (33,49)	25 (29,21)	28 (31,35)
Unscarified seeds	13 (20,20)	30 (31,96)	21 (26,08)
Mean	22 (26,85)	27 (30,58)	

Effects:	F Values:	LSD
Light	1,85	—
Scarification	3,68	—
Light× Scarification	8,50**	(10,55)

Effect of growth substances

Seeds were soaked in concentration series of gibberellic acid (GA_3; 0,1–1 000 ppm), indoleacetic acid (1–1 000 ppm), kinetin (3–48 ppm) and ethrel (1 000–5 000 ppm active ingredient). With the exception of ethrel, no significant promotion of germination was found. The optimal concentration of ethrel was

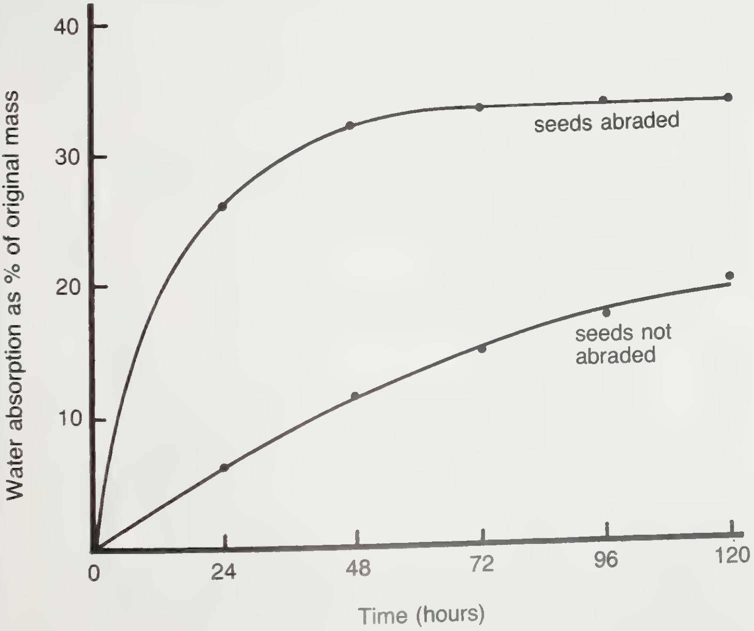

FIG. 1

Effect of abrasion on water absorption by *S. reginae* seeds.

TABLE 2

Germination† of *S. reginae* seeds treated with various concentrations of thiourea, urea, various mercaptans and ascorbic acid

Thiourea			Urea		Glutathione			Cysteine		BAL		Ascorbic acid		
Concentration		Transformed	Concentration	Transformed	Concentration		Transformed	Concentration	Transformed	Concentration	Transformed	Concentration		Transformed
ppm	M× 6,6	%	M	%	ppm	M× 3,25	%	M	%	M	%	ppm	M× 5,7	%
0	0	20,96	0	27,91	0	0	29,65	0	33,69	0	16,73	0	0	30,25
5	10^{-5}	16,72	10^{-5}	20,97	0,1	10^{-7}	31,47	10^{-4}	33,69	10^{-5}	11,67	0,1	10^{-7}	27,00
50	10^{-4}	34,02	10^{-4}	20,97	1	10^{-6}	35,23	10^{-3}	51,43	10^{-4}	21,62	1	10^{-6}	27,36
500	10^{-3}	48,20	10^{-3}	21,71	10	10^{-5}	33,63	10^{-2}	54,41	10^{-3}	36,23	10	10^{-5}	31,35
5 000	10^{-2}	44,20	10^{-2}	23,89	100	10^{-4}	45,70			10^{-2}	26,42	100	10^{-4}	37,24
50 000	10^{-1}	0,0	10^{-1}	0,0	1 000	10^{-3}	51,29					1 000	10^{-3}	58,76
F values:		75,31**		15,50**			8,90**		22,56**		7,34**			34,95**
LSD:		9,03		11,18			12,67		9,87		15,21			9,13

†Arcsin transformation of germination percentage

found to be 2 000 ppm. At this concentration 65 % of the seeds germinated as compared to 27 % in the control.

Effect of carbon dioxide and oxygen

The treatments applied in these experiments are listed in Table 3 and are expressed in terms of volumes of CO_2 and O_2 in the flasks as a percentage of the total gas volume.

TABLE 3

Effect of different carbon dioxide and oxygen concentrations on germination of *S. reginae* seeds (Arcsin transformed values in brackets)

Carbon dioxide		Oxygen	
Concentration %	Germination %	Concentration %	Germination %
0 (KOH in centre well)	3	20,0 (air)	2 (4,66)
		24,1	2 (4,66)
0,03 (air)	3	27,9	3 (8,51)
2,5	2	34,7	10 (18,09)
4,8	2	100,0	42 (40,24)
9,3	2		
17,4	2		

F value: 29,74**
LSD: (11,48)

The results of these experiments should be interpreted with caution as the seeds in the different treatments were exposed to different gas pressures. Respiratory activity of the seeds could also have changed the original gas composition.

In spite of these technical restrictions it seems valid to conclude from the results in Table 3 that whereas elevated CO_2 concentrations elicited no response, elevated O_2 concentrations had a stimulatory effect on germination. Poor germination in the control and CO_2 treatments was probably the result of limiting oxygen in the sealed flasks.

The beneficial effect of oxygen was confirmed in a subsequent experiment using larger flasks (600 mℓ capacity). Incubation in 100 % oxygen resulted in 64 % of the seeds germinating within one week. Seeds in control flasks that were not sealed, but stoppered with cotton-wool, showed a germination of only 11 %.

Mechanical restriction of embryo growth

Scarified seeds were allowed to imbibe water for two days after which the tissues covering the embryo in the proximal region of the seed were excised. As sterile conditions were difficult to maintain during this so-called chipping operation, the seeds were not sterilized. The seeds were incubated in quartz sand kept moist with daily applications of tap water. After three weeks, only eight per cent of the seeds in the control treatment had germinated in comparison with the 53 % germination attained by chipped seeds.

Effect of different storage conditions

Air-dry seeds were kept in cotton-wool stoppered flasks for four weeks at room temperature (*ca*. 20 °C), 40 °C, 50 °C as well as in sealed 600 mℓ flasks (at *ca*. 20 °C) flushed weekly with pure oxygen. A stratification treatment (imbibed seeds kept at 4 °C for four weeks) was also included.

None of the treatments resulted in an increased germination. Both storage at 50 °C and stratification proved to be decidedly deleterious, depressing germination by 21 and 34 %, respectively, relative to the room-temperature control.

A subsequent observation on air-dry seeds that had been stored for 10 months at 4 °C, showed that this treatment decreased germination by 18 % in comparison to laboratory-stored seeds.

Effect of oxidizing agents and respiratory inhibitors

Treatments consisting of concentration series of KNO_2 (1–1 000 ppm), KNO_3 (1–10 000 ppm), H_2O_2 (2–8M with different exposure times), methylene blue (10^{-4}–10^{-2}M) and KCN (10^{-5}–10^{-2}M) failed to show any stimulatory effect on germination. Germination was improved by a concentration of 10^{-4}M sodium azide, but the effect was not statistically significant.

Effect of leaching

Although there is evidence for the presence of a water-soluble inhibitor in the seeds of *S. reginae* (van de Venter & Small, 1975), leaching of scarified seeds in running tap water for six days failed to improve germination.

DISCUSSION

Judging from the variety of treatments which have been found to improve germination of *S. reginae* seeds, their dormant condition seems to be a complex phenomenon with possibly more than a single causative factor. It would be premature to speculate on possible control mechanisms at this stage but, nevertheless, the results have implicated the involvement of various factors which might be investigated more fully.

Thiourea, a well-known dormancy-breaking agent (Mayer & Poljakoff-Mayber, 1963) was found to improve germination of *S. reginae* seeds. The fact that various mercaptans, in contrast to urea, were able to improve germination, suggests that the stimulatory effect of thiourea resulted from the tendency of this compound to form a thio-imido tautomer which occurs in equilibrium with the normal form of thiourea (Toole, Hendricks, Borthwick & Toole, 1956; Garrard & Biggs, 1966). That the reductive capacity of these compounds is involved, was suggested by the positive results with ascorbate.

Paradoxically, the promotive effects of increased oxygen concentrations on germination indicate the necessity of an oxidation reaction for the termination of dormancy. Alternatively this result could simply be indicative of a permeability barrier in the seed coat. The magnitude of the response to oxygen points to a major involvement of this gas in the termination of dormancy and indicates that this factor should be further investigated.

Roberts (1969, 1973) proposed that, in the seeds of certain species, cytochrome oxidase competes for available oxygen with a dormancy-breaking reaction. He postulated that operation of the pentose phosphate pathway is a prerequisite for the termination of dormancy. The seeds of *S. reginae*, although responding positively to oxygen and thiourea, failed to do so to electron acceptors, respiratory inhibitors, storage in elevated oxygen tension, and stratification. These results suggest that the mechanism, as proposed by Roberts, would not serve as a hypothetical model for dormancy in *Strelitzia* seeds.

The view that dormancy is essentially under hormonal control, that is, inhibitors interacting with growth promoters, has been expounded by authors such as Mayer (1960) and Amen (1968). Control of this nature in the case of *Strelitzia* is a distinct possibility and merits further attention. Evidence for the presence of an inhibitor has been presented (van de Venter & Small, 1975) and the involvement of ethylene in the termination of dormancy (as implied by the effect of ethrel) is indicated by these results.

Possible correlations between the factors already mentioned and the mechanical restriction of embryo growth by the seed coat are not clear at this stage. Mechanical restriction by the seed coat is known to be involved in the seed dormancy of various species (Randolph & Cox, 1943; Chen & Thimann, 1964, 1966; Chen, 1968).

The significance of the light x scarification interaction is also vague. Mayer & Poljakoff-Mayber (1963) have reported that seed-coat damage can alter the response of seeds to light.

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